nonsymbiotic plant hemoglobin is barley nonsymbiotic hemoglobin.--

Copies of specification pages 26-27, amended in accordance with the claim amendments described above, are enclosed.

REMARKS:

As the examiner can see, the claims have been amended to state that the hemoglobins used are nonsymbiotic plant hemoglobins.

The previous claims were rejected under 35 U.S.C. 102(e) as being anticipated by Bailey (US Patent 5,959,187) and under 35 U.S.C. 102 (b) as being anticipated by Bailey (WO 98/12913).

Applicants note that US Patent 5,959,187, column 2, lines 60-66 and WO 98/12913, page 3 lines 28-32 states "the few plant hemoglobin-like molecules described, which include lupin and soybean leghemoglobin, are largely thought to be associated with nitrogen-fixation activities of these plants, although some researchers claim that hemoglobin-like proteins occur in the roots of all plants. However, in no case have these proteins been overexpressed in plants (emphasis added)".

It is further noted that in neither reference does Bailey demonstrate the overexpression of plant hemoglobins in plants. Rather, Bailey demonstrates the expression of a bacterial hemoglobin gene from *Vitreoscilla* in plants.

In the examples of "gene targets", Bailey lists soybean leghemoglobin and lupin hemoglobin (see for example US Patent 5,959,187, column 4, lin s 51-52) and also states that "particularly suitable for use in the pres nt invention ar

those oxygen-binding proteins which have relatively high k_{off} rates such as VHb (k_{off} 5600 s^{-1}...) or relatively low oxygen affinity such as horse heart myoglobin (K_D 0.79 μM...)".

As discussed on page 1 of the instant application, the "nonsymbiotic hemoglobins ... are thought to be the evolutionary predecessors of the more specialized symbiotic leghemoglobins". Furthermore, as discussed on page 2 of the instant application, the nonsymbiotic hemoglobins have high oxygen avidity", not low oxygen affinity. In addition, the nonsymbiotic hemoglobins have relatively low k_{off} rates approximately 150 times lower than *Vitroscilla* hemoglobin (Giangiacomo et al., *Biochemistry* **40**: 9311-9316). Thus, the cited references teach against the use of nonsymbiotic plant hemoglobins as these proteins have very different properties compared to the symbiotic plant hemoglobins described by the Bailey references.

Thus, US Patent 5,959,187 and WO 98/12913 do not teach or suggest applicant's invention in that neither references teaches or suggests the overexpression of nonsymbiotic plant hemoglobins in plants. In fact, these references admit that plant hemoglobins have not been overexpressed in plants, as discussed above. Finally, as discussed above, the cited references in fact teach against the use of nonsymbiotic plant hemoglobins. In view of this and the amendments to the claims, it is respectfully requested that the examiner reconsider these rejections.

The cancellation of claims 23 and 24 to exclude certain subject matter is made without prejudice and Applicants have no intention at this tim to abandon

that subject matter. Applicants hereby expressly reserve right t pursue the same or similar subject matt r in a continuing application.

In view of the foregoing, further and more favorable consideration is respectfully requested.

Respectfully submitted

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CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that this paper is being facsimile transmitted to the Patent And Trademark Office on the date shown below.

MICHAEL WILLIAMS

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DATE: November 30, 2001

CLAIMS

28. A method of improving the agronomic properties of a plant comprising:

providing a plant having increased cellular levels of a nonsymbiotic plant hemoglobin; and

growing the plant.

29. The method according to claim 28 wherein the nonsymbiotic plant hemoglobin is barley nonsymbiotic hemoglobin.

30. The method according to claim 28 wherein the improved agronomic properties include germination.

31. The method according to claim 28 wherein the improved agronomic properties include seedling vigour.

32. The method according to claim 28 wherein the improved agronomic properties include reduced cellular levels of fermentation products.

33. The method according to claim 28 wherein the improved agronomic properties include increased oxygen uptake.

34. The method according to claim 28 wherein the improved agronomic properties include increased tolerance to hypoxic conditions.

35. A method of selecting seeds for breeding to produce seed lines having desirable characteristics comprising:

providing a representative seed of a given seed line;

growing the seed such that the seed germinates;

isolating an extract from the se d;

measuring levels of nonsymbiotic plant hemoglobin xpression

within the extract; and

selecting or rejecting the seed for further breeding based on the hemoglobin levels.

36. The method according to claim 35 wherein the nonsymbiotic plant hemoglobin is barley nonsymbiotic hemoglobin.

37. A method of determining if a seed is germinating comprising:

providing a seed suspected of germinating;

isolating an extract from the seed; and

measuring levels of nonsymbiotic plant hemoglobin expression within the extract,

wherein high levels of nonsymbiotic plant hemoglobin expression indicate that the seed is germinating.

38. The method according to claim 37 wherein the nonsymbiotic plant hemoglobin is barley nonsymbiotic hemoglobin.